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A new algal book, described below, will be published in December 1998 by the Muséum national d'Histoire naturelle (Paris). A **print run of only 400 copies is scheduled**. Consequently, those interested should order as soon as possible to ensure getting a copy. The book is written in English and contains vi + 767 pages. The 369 photographic and lithographic figures include examples of the handwritings of 54 persons and portraits of 39 persons, and thus the publication is likely to be of wider interest as a general reference than the title might suggest. Brief biographic sketches of over 150 persons are also included along with considerable historical information on algal research directly or indirectly involving the Paris Muséum.

**Orders for and inquiries about the book should be sent to: Association des Amis des Cryptogames, 12 rue Buffon, Paris 75005, France.**

The ordinary price of the book is 1200 French francs (postage included), but subscribers (both individuals and institutions) to the journal *Cryptogamie, Algologie* will be given a 50 % discount. Payment should NOT be included with orders; invoices will be sent after orders are processed.

## BOOK DESCRIPTION (information from back cover)

Non-geniculate coralline red algae are historically and biologically fascinating organisms of considerable ecological importance. Their taxonomy has been attended by a particularly confused nomenclature, and their scientific history has been full of controversy and debate. The Muséum national d'Histoire naturelle, in Paris, houses one of the most extensive and taxonomically and historically important collections of these algae in the world, including the original specimens of J.-B. Lamarck (*ca* 1780) to M. Lemoine (*ca* 1980) whose types are represented at the Muséum. It also includes historical analyses of French-based and French-influenced studies of these algae in the context of the development of botanical research at the Muséum. In addition, brief biographic notes are provided for over 150 other persons who collected specimens now housed at the Muséum. With over 1000 bibliographic references and 369 photographic and lithographic plates, this book represents one of the most significant publications on non-geniculate coralline algae to date and is destined to remain a standard reference for phycologists, curators, and historians of science.



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## ALGOLOGIE

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## A STUDY OF *PADINA ANTILLARUM* (KÜTZING) PICCONE AND A COMPARISON WITH *P. TETRASTROMATICA* HAUCK (DICTYOTALES, PHAEOPHYTA)

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**ABSTRACT** — Holotype material of *Zonaria antillarum* Kützinger [= *Padina antillarum* (Kützinger) Piccone] was located in MEL and examined. The provenance of this poorly known taxon is Trinidad in the West Indies. A comparison of this alga is made with other species of *Padina*, and the conclusion is made that it is a taxonomic synonym of *P. tetrastrumatica* Hauck. The priority of *Zonaria antillarum* Kützinger (1859) over *P. tetrastrumatica* Hauck (1887) necessitates the replacement of the latter name with *P. antillarum* (Kützinger) Piccone. A listing of currently recognized species of *Padina* is provided.

**RÉSUMÉ** — L'holotype de *Zonaria antillarum* Kützinger [= *Padina antillarum* (Kützinger) Piccone] a été localisé à MEL et examiné. Ce taxon mal connu provient de Trinidad, aux Antilles. Cette algue a été comparée aux autres espèces de *Padina* et a été mise en synonymie avec *P. tetrastrumatica* Hauck. La priorité de *Zonaria antillarum* Kützinger (1859) sur *P. tetrastrumatica* Hauck (1887) implique de remplacer le second par *P. antillarum* (Kützinger) Piccone. Une liste des espèces de *Padina* actuellement reconnues est aussi fournie dans cet article. (Traduit par la Rédaction)

**KEY WORDS:** marine algae, *Padina*, *P. antillarum*, *P. tetrastrumatica*, Phaeophyta, *Zonaria antillarum*.

### INTRODUCTION

*Zonaria antillarum* Kützinger (1859) has remained a poorly known taxon of brown algae of unspecified provenance and uncertain status. Although the specific epithet refers to the Antilles of the tropical western Atlantic, the species was not included in the floristic treatment of this region by Taylor (1960) nor picked up by Wynne (1986) in his catalogue of algae for this same domain. Its inclusion, as *Padina antillarum* (Kützinger) Piccone (1886), in the recent catalogue of Indian Ocean marine algae by Silva *et al.* (1996) came to my attention as well as fact that its type locality had not been specified by Kützinger (1859). The basis for the Indian Ocean record was Piccone's (1886, 1889) report of its presence from Singapore as well as his transfer of this species from *Zonaria* to *Padina*. The

discovery of the Holotype collection of this alga in the National Herbarium of Victoria (MEL), Melbourne, allowed for its examination and a clarification of its status.

In Vol. 9 of his *Tabulae phycologicae* Kützinger (1859) described a number of new species of Dictyotales from various locations around the world, including some from the western Atlantic (Table 1), on the basis of specimens in the Sonder Herbarium. Following the death of Sonder, Ferdinand Mueller, founder of the National Herbarium of Victoria, purchased the Sonder Herbarium from Sonder's nephew (Ducker, 1981a, 1981b), which explains how many of Sonder's types came to reside in MEL.

Table 1. Taxa of Dictyotales newly described from the western Atlantic by Kützinger (1859).

Taxon	Provenance	Currently accepted name
<i>Dictyota antiquae</i>	Antigua, Lesser Antilles	<i>D. guineensis</i> (Kützinger) P. Crouan & H. Crouan
<i>D. cervicornis</i>	Key West, Florida	<i>D. cervicornis</i>
<i>D. ciliolata</i>	La Guaira, Venezuela	<i>D. ciliolata</i>
<i>D. cuspidata</i>	Veracruz, Mexico	<i>D. cuspidata</i>
<i>D. indica</i>	Havana, Cuba	<i>D. cervicornis</i>
<i>D. pardalis</i>	« E mari Antillarum » [« Senegambia » on type sheet]	<i>D. cervicornis</i>
<i>D. pinnatifida</i>	Antigua, Lesser Antilles	<i>D. pinnatifida</i>
<i>Dictyota subdentata</i>	Antilles Lesser Antilles	<i>D. mortensii</i> (Martius) Kützinger
<i>Spotoglossum versicolor</i>	Brazil	<i>Stypopodium zonale</i> (Lamouroux) Papenfuss
<i>Zonaria gymnospora</i>	St. Thomas, U. S. Virgin Islands	<i>Padina gymnospora</i> (Kützinger) Sonder
<i>Zonaria antillarum</i>	Provenance not cited	<i>Padina antillarum</i> (Kützinger) Piccone

*Zonaria antillarum* has remained of uncertain status. J. Agardh (1882) regarded *Z. antillarum* along with Kützinger's *Z. gymnospora* and his concept of *Z. variegata* as taxonomic synonyms of *Padina durvillaei* Bory de Saint-Vincent. Piccone (1886, 1889) transferred the species to *Padina* while recording it from Singapore; he made no reference to J. Agardh's (1882) treatment. De Toni (1895) and Weber-van Bosse (1913) made passing reference to *Z. antillarum*, simply accepting J. Agardh's interpretation. Børgesen (1914) stated that J. Agardh was wrong in referring *P. antillarum* to *P. durvillaei* but did not indicate how he would treat it. Neither Murray (1888-1889) nor Taylor (1960) made reference to *Zonaria* (or *Padina*) *antillarum* for the Caribbean. In her unpublished dissertation Thivy (1945) discussed the species on the basis of Kützinger's original description and figures and stated that *Zonaria antillarum* probably applied to a *Zonaria* and not to a *Padina*. Her reason for this idea was that the sori of Kützinger's plant were described as consisting of clusters of sporangia with hairs arising among the sporangia as in *Zonaria*. Yet Thivy recognized that in *Zonaria* an indusium covers the sori, whereas an indusium was not figured for Kützinger's plant. The sporangial sori of *Zonaria* often bear sporangia intermixed with multicellular paraphyses (Allender & Kraft, 1983), but this feature is not characteristic of all species in the genus (Womersley, 1987; Phillips, 1997). Teo & Wee (1983) reported three species of *Padina* from Singapore: *P. gymnospora* (Kütz.) Sond. (erroneously as a species with distromatic thalli, and thus probably *P. boergesenii* Allender & Kraft), *P. boryana* Thivy (as *P. tenuis*), and *P. tetrastromatica* Hauck, but they made no reference to Piccone's (1886) earlier record of *P. antillarum* from there.



## MATERIALS AND METHODS

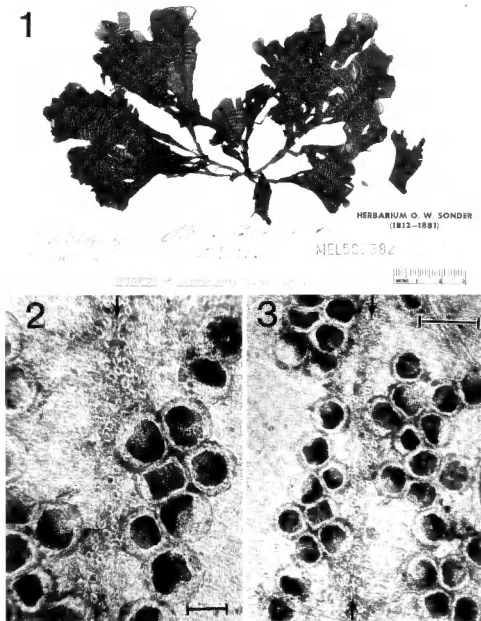
The holotype of *Zonaria antillarum* was received on loan from MEL (No. 583382). Small portions of the specimen, including distal, mid-blade, and proximal regions of the blade were rehydrated, gently cut away and mounted on glass slides for observation with a standard Zeiss research microscope. Some portions of blade were hand-sectioned using a single-edged razor blade. Line-drawings were made with a camera lucida attached to the microscope, and photomicrographs, using Kodak T-MAX 100 film, were made with a camera-back attached to the same microscope. Abbreviations of herbaria follow Holmgren *et al.* (1990).

## OBSERVATIONS

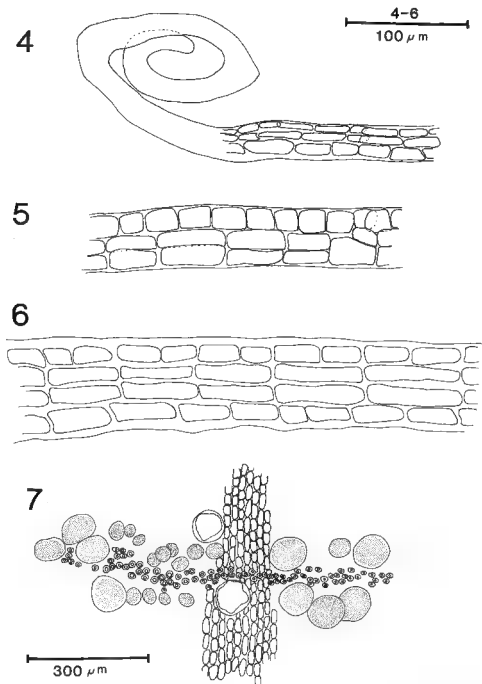
The holotype specimen of *Zonaria antillarum* (Fig. 1) is labeled "*Padina Pavonia* Lx", "Trinidad", and "Krüger" in Sonder's hand and "*Zonaria Antillarum* Kg." "Tab. ph. IX. Tab. 72." in Kützinger's hand. Mrs Doris Sinkora, former algal curator at MEL, has added (dated 19.iii.1981) to the herbarium sheet the information that the collector "Hermann Krüger (1818-1864) was born in Hamburg, went to live in Trinidad in 1841 and changed the spelling of his name to Crüger, was Colonial Botanist & Superintendent of the Botanic Garden there [from] 1857 till his death in 1864". This information was derived from Urban (1902).

Kützinger (1859, Tab. 72, fig. II) depicted the habit of the plant as deeply divided into cuneate to flabellate segments; a cross-section of the lower part of the thallus, which is four cell-layers in thickness, with hair-forming articulated filaments, which constitute the stipe; a mid-thallus cross-section, three cell-layers in thickness, with sporangia and short secondary filaments interspersed among the sporangia; and a surface view of the thallus showing sporangia and secondary filaments, the latter viewed from the top.

The holotype specimen consists of a number of flabellate segments reaching 9 cm in length and deeply divided to the base. The narrow basal parts are densely covered with rhizoids. Individual segments are 2.0-3.0 cm broad. There is no obvious calcification. The circinnately inrolled margin (Fig. 4) confirms the fact that it is a *Padina*, not a *Zonaria* as Thivy (1945) suggested. Sagittal sections of the thallus in the distal region show that it is three cell layers in thickness (Fig. 5), whereas sections of the thallus in mid-blade show four cell layers (Fig. 6). Almost all segments are fertile, bearing bands of sori on only the inferior surface of the blade, *i. e.*, the surface of the blade opposite the side toward which the margin is curled. Soral bands appear close to the growing margin, and they are regularly spaced at a distance of 1.0-1.6 mm. The prominence and thickness of the soral regions give the blades a distinctly corrugated aspect. The division of some of the reproductive organs indicates that they are tetrasporangia rather than oogonia. Mature sporangia are 70-90 µm in diameter. There is no indication of an indusium over mature sori. The sporangia closely abut a continuous line of hairs (a piliferous zone) (Figs 2, 3, & 7). Typically, all that remains of the hairs are the basal cell remnants. Paraphyses of the type that occurs in *Zonaria* were not present. The short multicellular filaments shown by



Figs 1-3. *Padina antillarum* (based on holotype of *Zonaria antillarum* in MEL). Fig. 1. Habit. Figs 2 & 3. Surface view of soral regions, showing sporangia lying on both sides of zone of hairs (arrows). Scale bars in Figs 2 & 3 = 50 µm.



Figs 4-7. *Padina antillarum* (based on holotype of *Zonaria antillarum*). Fig. 4. Sagittal section of apical region of blade showing circinnately inrolled margin. Fig. 5. Sagittal section of distal region of thallus. Fig. 6. Sagittal section of mid region of thallus. Fig. 7. Schematic surface view of sororal region, showing arrangement of sporangia on either side of zone of hairs.

Kützling (1859, pl. 72, fig. 11, c) are merely hairs. The sporangia are densely arranged along both sides of this zone of hairs (Figs 2 & 3), at times giving the impression of being intermixed with the hairs, as Kützling (1859) depicted them.

The characteristics seen in the holotype of *Zonaria antillarum* present a persuasive case that it is the same as *Padina tetrastromatica* Hauck. According to Jaasund (1976) *P. tetrastromatica* is easily recognized because of the double lines of sporangia on either side of a line of colorless hairs and because it is the only East African species of *Padina* that occurs on open shores with surf activity, which explains the tendency of the thallus to split into narrow segments. Other hallmark features of *P. tetrastromatica* are that thalli are rarely encrusted with limestone, that tetrasporangial sori are non-indusiate, and that thalli are primarily four cell layers in thickness. All of these characteristics are shared by *P. antillarum* and *P. tetrastromatica*, pointing to their taxonomic identity. Evidence for the conspecificity of these two taxa will be presented in the Discussion, especially in reference to the observations made on *P. tetrastromatica* by Gaillard (1967).

Lawson & John (1987) described thalli of West African *Padina tetrastromatica* to be often distinctly corrugated, to be repeatedly divided into delicate and narrow segments, uncalcified, with the zones of hairs 1-1.5 mm apart; thallus four cells thick throughout; sori developing close to and on either side of each hair zone, without an indusium. This description closely matches the above account of the holotype of *Zonaria antillarum*.

## DISCUSSION

In Taylor's (1960) comprehensive flora of the eastern tropical and subtropical coasts of the Americas, six species of the brown algal genus *Padina* were recognized. Two of these species (*P. haitiensis* and *P. perindusiata*) were newly described and credited to Francesa Thivy, whose doctoral dissertation had been completed at the University of Michigan in 1945. She was also credited with the new combination *Padina pavonica* (Linnaeus) Thivy to replace the incorrect name *P. pavonia* (Linnaeus) Lamouroux. Another Caribbean species was *P. vickersiae* Hoyt in Howe (1920), which had been the alga depicted as *Zonaria variegata* (Lamouroux) C. Agardh by Kützling (1859, pl. 73) and as *Padina variegata* (Lamouroux) Hauck by Vickers (1908, pl. VIII) and by Børgesen (1914, figs 157-161). Børgesen (1920) had applied *P. howeana* nov. nom. to this same entity.

In the ensuing years following Taylor's (1960) treatment the list of species of *Padina* has been altered. Gaillard (1975) suggested that *P. haitiensis* was conspecific with *P. sanctae-crucis* Børgesen, but due to the scarcity of specimens she refrained from making a formal proposal to merge the two taxa. So *P. haitiensis* continues to be recognized. Taylor's (1960) observation that *Dictyterpa jamaicensis* Collins (1901) was "a growth stage of a *Padina*, perhaps of various species, but certainly of [*P. sanctae-crucis*]" caused Papenfuss (1977) to propose *P. jamaicensis* (Collins) Papenfuss as the correct name. But because of the uncertainty which species the type of *D. jamaicensis* corresponded to, Silva *et al.* (1987) preferred to retain the name *P. sanctae-crucis*. Earle (1969) described *P. profunda* as a new species from the Gulf of Mexico. Although *P. boryana* Thivy in Taylor and *P. caulescens* Thivy in Taylor were included in Wynne's (1986) checklist, no basis could later be found for their inclusion, and they have been deleted from the checklist revision (Wynne, 1998).

In his floristic account of the marine algae of Trinidad, Richardson (1975) listed only two species of *Padina*: *P. vickersiae* and *P. gymnospora*. Allender & Kraft (1983) clarified previous taxonomic confusion concerning these two names. Most authors (Børgesen, 1914; Taylor, 1960; Earle, 1969) had misinterpreted *P. gymnospora* to be a tristromatic species, even though Børgesen (1914) appreciated the fact that this interpretation was in conflict with one of the figures given in Kützinger's (1859) plate 71. Allender & Kraft's (1983) examination of the type (*Zonaria gymnospora* Kützinger) in MEL showed it to be four cell layers in mid frond and six-eight cell layers near the base, which was in agreement with Kützinger's protologue. Thus, Allender & Kraft (1983) concluded that *P. vickersiae* must be treated as a taxonomic synonym of *P. gymnospora* and that a new species, *P. boergesenii* Allender & Kraft, was to be recognized for the tristromatic species which had been mistakenly identified as *P. gymnospora*.

Womersley (1987) reported *Padina gymnospora* (in the sense of Allender & Kraft, 1983) from southern Australia and characterized it as four cells thick above and 6-8(-9) cells thick in mid and lower parts. According to Womersley an indusium over the sporangia is "absent or slight". In their account of this species from the southeastern coast of the United States, Schneider & Searles (1991) described the plants as becoming deeply lacinate, with cuneate-spatulate to fan-shaped segments. They said that the reproductive structures occurred in one or two bands midway between hairlines on both surfaces but primarily on the ventral surface. The oogonia were protected by a thin, evanescent covering of sterile cells, and the tetrasporangia also had sterile coverings.

Differences of opinion exist on the thallus thickness of *P. tetrastromatica*. In Hauck's (1887) original account of the species the thalli were described as three layers of cells near the apex and four (rarely six) layers toward the base. According to Thivy (1945, p. 184) *P. tetrastromatica* cannot be considered as having more than four layers of cells in the frond. When six or seven layers are present, they are found only in the "Flachtriebe" (= ligulate branch) of the germling which may persist at the base of the stipe of the adult plant. The "Flachtriebe" is distinguishable from the frond proper. Weber-van Bosse (1915) said that her plants from the East Indies were four cell layers thick from close to the apex down to the base. Børgesen (1936) reported on material from Ceylon to have four cell layers, while in material from India Børgesen (1930) observed 2 cell layers near the growing edge and three of four cell layers in the basal regions. Allender & Kraft (1983) referred to *P. tetrastromatica* as a "basically tristromatic species" which tended to become four layers thick near the base.

The most detailed description of *P. tetrastromatica* was provided by Gaillard (1967), who based her account on herbarium material including three syntype specimens (in L) and recent collections from Dakar, Senegal, West Africa. The specimen collected by Hildebrandt (Hauck 68) from Meith, Somalia, and depicted by Gaillard (1967, fig. 1) was designated the "Typus" according to P. Audiffred (pers. comm.). Gaillard found that the type specimens all presented a tetrastromatic structure. Gaillard observed that the specimens from West Africa were four layers of cells in their broadest part; only at the base could there be six cell layers.

Gaillard (1967) found that an indusium present in the early stages of tetrasporangial sorus formation was soon shed. All authors are in agreement that mature tetrasporangial sori lack an indusium. Gaillard observed the rows of reproductive organs to be almost contiguous to the hairs and that it is impossible to measure the distance between sporangia and hairs. Gaillard also confirmed Hauck's (1887) observation that some plants in this species can produce plantules, which arise in the same position as the tetrasporangial sori. Lawson & John (1977) also noted this same phenomenon in plants of this species from the Cap Blanc Peninsula of West Africa.

Although Allender & Kraft (1983) depict *Padina tetrastromatica* as bearing sporangia on only one surface of the thallus, Gaillard (1967) and Schnetter (1976) depict sporangia in this species as being sometimes produced on both blade surfaces. Gaillard described the sporangia to be arranged in sori abutting the hair zones on the inferior side, whereas on the superior blade surface the sporangia are borne at mid-distance between the soral bands on the opposite face, although they are most often poorly developed and the sporangia are intermixed with hairs instead of surrounding the hair bands. Gaillard observed this arrangement both in the syntype specimens and in those from Senegal. She also stated that in the majority of specimens of *P. tetrastromatica* from elsewhere, the reproductive organs are localized only on the inferior face and form only very sporadic groups on the other face. In MICH holdings of *P. tetrastromatica* the production of sporangia on both blade surfaces, as Gaillard (1967) depicted them, was observed in two West African collections (*leg. D. M. John 6430C* from Ghana; *leg. G. W. Lawson A1186* from the Gold Coast), but in numerous Philippine specimens sporangia were produced only on the inferior face. So this is regarded as a variation within the species.

No world monograph of *Padina* has been published in a modern context, but a survey of the literature has shown that 32 species are currently recognized. Data on these are summarized in Appendix 1. How many true biological species these represent remains to be determined.

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## Appendix 1. Currently Recognized Species of *Padina*

### 1) *P. antillarum* (Kützinger) Piccone, 1886, p. 36.

Basionym: *Zonaria antillarum* Kützinger, 1859, p. 29, pl. 72, fig. 11.

Type locality: not indicated. Holotype in MEL: Trinidad, Lesser Antilles, West Indies.

Tax. syn.: *P. tetrastromatica* Hauck, 1887, p. 43.

Type locality: Meith, Somalia. Lectotype in L (Hauck No. 68 = Gaillard, 1967, fig. 1).

Distribution: widely distributed in Indian Ocean, including East Africa, Yemen, Oman, Arabian Gulf, India, Pakistan, Ceylon; Philippines, Malaysia, Indonesia, northeastern Australia, China, West Africa, Colombia, Venezuela. According to Thivy (1945), the report of *P. tetrastromatica* by Setchell & Gardner (1930) from Pacific Mexico is most likely *P. crispata*.

References (all of the following were as *P. tetrastromatica*): Weber-van Bosse, 1913, pp. 180-181; Bergesen, 1930, p. 172, fig. 10, pl. II; Durairatnam, 1961, p. 36, pl. VII, figs 4.5, pl. XXVI, fig. 1; Gaillard, 1967, p. 447-463; Srinivasan, 1969, p. 26, pl. XXVI; Schnetter, 1976, p. 76, pl. IX, fig. 1, K; Pl. XI, fig. B; Jaasund, 1976, p. 45, fig. 90; Price *et al.*, 1978; Ngan & Price, 1979, p. 8, 1980, p. 186; Lawson & John, 1987, p. 131, pl. 15, figs 4 & 5; Tseng, 1983, p. 200, pl. 101, fig. 2; Allender & Kraft, 1983, p. 89, fig. 7F; Jones, 1986, p. 31, pl. 6; Ganesan, 1990; Shameel & Tanaka, 1992; Ormond & Banaimoon, 1994; Wynne & Luong-Van Thinh, 1997, p. 85; Wynne & Jupp, 1998, p. 11.

### 2) *P. arborescens* Holmes, 1896, p. 251, pl. 12, fig. 1.

Type locality: Enoshima, Japan. Holotype in BM.

Distribution: Japan, Korea, Fujian and Guangdong provinces of China, Hong Kong, Taiwan, and the Philippines.

References: Okamura, 1929, p. 3, pl. 251, fig. 10, pl. 252; Yamada, 1931, p. 67; Lee, 1964, p. 53, pl. 7, fig. 2; Chihara, 1970, p. 24, pl. 12(1); Tseng, 1983, p. 196, pl. 99, fig. 4; Lewis & Norris, 1987, p. 12; Silva *et al.*, 1987, p. 77; Kajimura, 1996, p. 10, figs 1-16.

### 3) *P. australis* Hauck, 1887, p. 44.

Type locality: Cape York, northern Australia (earlier treated as *P. gymnospora* by Sonder, 1871). Holotype in L.

Distribution: Japan, Philippines, Hong Kong, Taiwan, Thailand, Indonesia, Malaysia, Papua New Guinea, Lord Howe Island, eastern and northern Australia, New Zealand ("?"), and West Africa.

References: Yamada, 1931, p. 70, text-fig. 2 and pl. XVIII; Okamura, 1932, p. 88, pl. 295, figs 1-4; Lee, 1964, p. 53, pl. 6, fig. 6, and pl. 7, fig. 1; Egerod, 1974, p. 151, fig. 85; Magruder & Hunt, 1979, p. 47; Trono & Ganzon-Fortes, 1980, p. 41, fig. (s. n.); Allender & Kraft, p. 85, figs 5 C, 6 B; Tseng, 1983, p. 198, pl. 100, fig. 1; Lewis & Norris, 1987, p. 12; Lawson & John, 1987, p. 128, pl. 15, fig. 9; Farrant & King, 1989, p. 388, fig. 12; Adams, 1994, p. 89; Coppejans *et al.*, 1995, p. 184, fig. 22; Calumpong & Meñez, 1997, p. 126.

var. *cuneata* Tanaka & Nozawa, 1962, p. 179, pl. 1, A, fig. 1.

Type locality: Amadomari, Nishino-omote, Tanegashima, Japan. Holotype presumably in KAG.

### 4) *P. boergesenii* Allender & Kraft, 1983, p. 87, figs 6 C, H & I, 7 C & D)

Type locality: Danish West Indies [= U. S. Virgin Islands]. Holotype in MEL.

Distribution: broadly distributed in tropics. According to Allender & Kraft (1983) voucher material needs to be examined to determine whether various records of *P. gymnospora* [e.g., Thivy (1959) from India and Taylor (1966) from the Philippines] refer to *P. boergesenii*.

References: Vickers, 1908, p. 37, pl. VII (as *P. gymnospora*); Bergesen, 1914, p. 202, figs 155, 156 (as *P. gymnospora*); Taylor, 1960, p. 237 (as *P. gymnospora*); Sartoni, 1975, p. 289, figs 9 & 11 (as *P. gymnospora*); Schnetter, 1976, p. 73, pl. IX, fig. H; pl. XI, fig. A (as *P. gymnospora*); De Clerck & Coppejans, 1996, p. 230, figs 48, 50-51; González-González *et al.*, 1996, p. 94.

**5) *P. boryana* Thivy in Taylor, 1966, p. 355, fig. 2.**

Type locality: "Friendly Islands" = Tonga, Polynesia. Holotype in NY.

Distribution: tropical Indian and Pacific Oceans, West Africa.

Misapplied name: *Padina commersonii* Bory de Saint-Vincent (1828). See Silva *et al.* (1996, p. 603), who explain that *P. commersonii*, a name that had been traditionally applied to the present taxon, is an illegitimate substitute for *P. tenuis* (C. Agardh) Bory de Saint-Vincent (1827). The basionym of this latter name, *Zonaria pavonia* var. *tenuis* C. Agardh (1824) was demonstrated by Womersley & Bailey (1970) to be the same as *Lobophora variegata* (Lamouroux) Womersley *ex* Oliveira.

References: Okamura, 1932, p. 89, pl. 295, figs 5-11 (as *P. commersonii*); Tsuda, 1972, p. 98, pl. 5, fig. 4 (as *P. tenuis*); Levring, 1943, p. 176, fig. 1 B-F; Tanaka & Nozawa, 1962, p. 180, pl. II, A, fig. 2 (as *P. commersonii*); Egerod, 1974, p. 150, fig. 84 (as *P. tenuis*); Kapraun & Bowden, 1978, p. 200 (as *P. tenuis*); Allender & Kraft, 1983, p. 83, figs 5 D & E, 6 A (as *P. tenuis*); Tscng, 1983, p. 198, pl. 100, fig. 2; Lawson & John, 1987, p. 130; Farrant & King, 1989, p. 390, fig. 15 (as *P. tenuis*); Tsuda, 1991, p. 45; Coppejans *et al.*, 1995, p. 188.; N'Yeurt, 1996, p. 402, fig. 108f (as *P. tenuis*).

**6) *P. caulescens* Thivy in Taylor, 1945, p. 99.**

Type locality: off Isla Maria Magdalena, Las Tres Marias, Nayarit, México. Holotype in LAM.

Distribution: Pacific Mexico and Gulf of California.

References: Dawson, 1959, p. 18; Chavez, 1980, p. 48; Mendoza-Gonzalez & Matco-Cid, 1986, p. 421; González-González *et al.*, 1996, p. 155.

**7) *P. conrescens* Thivy in Taylor, 1945, p. 102.**

Type locality: Black Beach Anchorage, Isla Santa Maria, Galapagos Islands. Holotype in LAM.

Distribution: Galapagos Islands, Pacific Mexico.

Reference: González-González *et al.*, 1996, p. 155.

**8) *P. crassa* Yamada, 1931, p. 67, pl. XVII, fig. 2.**

Syntype localities: Sagami Prov., Iyo Prov., Nagato Prov., Chikuzen Prov., Hizen Prov., Mutsu Prov., Izumo Prov. Holotype presumably in SAP.

Distribution: China, Japan, Korea, Hong Kong, eastern Australia including Lord Howe Island.

References: Okamura, 1932, p. 87, pl. 294, figs 5-11; Lee, 1964, p. 53, pl. 6, fig. 3; Chihara, 1970, p. 24, pl. 12 (2); Allender & Kraft, 1983, p. 87, figs 6 G, 7 A & B; Tseng, 1983, p. 198, pl. 100, fig. 3; Farrant & King, 1989, p. 388, fig. 13; Tsuda, 1991, p. 45; Kajimura, 1993, p. 19, figs 1-17.

**9) *P. crispata* Thivy in Taylor, 1945, p. 100.**

Type locality: Golfo Dulce, Costa Rica (Pacific coast). Holotype in LAM.

Distribution: Pacific Central America, Pacific Mexico, Pacific Colombia

References: Chavez, 1980, p. 47; Schnetter & Bula Meyer, 1982, p. 64, pl. 10, fig. H; Mateo-Cid & Mendoza-González, 1991a, p. 24; González-González *et al.*, 1996, p. 156.

**10) *P. distromatica* Hauck, 1887, p. 43.**

Type locality: Meith, Somalia (a Hildebrandt collection). Holotype in L.

Distribution: East Africa, Seychelles, Indonesia, Philippines.

References: Sartoni, 1975, p. 289, fig. 10.

**11) *P. dubia* Hauck, 1887, p. 45.**

Type locality: Meith, Somalia. Holotype in L.

Distribution: East Africa, Pakistan, India.

References: Sacco, 1965, p. 550, pl. XXXIII; Panikkar & Chauhan, 1991a, p. 139, figs 1-35, & 1991b, p. 149, 1-22.

**12) *P. durvillaei* Bory de Saint-Vincent, 1829, p. 147, Atlas, 1826, pl. 21, fig. 1.**

Type locality: Concepcion, Chile. Syntype material in PC.

Distribution: Pacific Mexico, Gulf of California, Mexico; Pacific Panama and Costa Rica; Pacific Colombia; Galapagos Islands; tropical West Africa.

References: Taylor, 1945, p. 101; Schnetter & Bula Meyer, 1982, p. 65, pl. X, fig. 1; Lawson & John, 1987, p. 130, pl. 15, figs 6, 7; González-González *et al.*, 1996, p. 156.

forma *obscura* Piccone, 1886, p. 36.

Type locality: Rio de Janeiro, Brazil. Holotype presumably in VER.

**13) *P. elegans* Koh ex Womersley, 1987, p. 220, figs 74B, C, 75K-M.**

Type locality: Muldrup Reef, Cottesloe, Western Australia. Holotype in UWA.

Distribution: Port Denison, Western Australia, to Pearson Island, South Australia.

**14) *P. fernandeziana* Skottsberg & Levring in Levring, 1941.**

Syntype localities: numerous stations listed in the Juan Fernandez Islands, Chile. Holotype in GB.

Distribution: as above.

References: Etcheverry, 1986, p. 95, pl. III.

**15) *P. fraseri* (Greville) Greville, 1830, p. xlv.**

Basionym: *Zonaria fraseri* Greville, 1829, p. 423 & 424, pl. XXVI.

Type locality: New Holland = Australia [New South Wales or Victoria according to Womersley, 1987, p. 218]. Holotype in *E. fide* Womersley (1987).

Tax. syn.: *P. tristromatica* Levring, 1942 p. 60, figs 1 & 2; syntype localities: San Felix and San Ambrosio, Juan Fernandez Islands, Chile (*fide* Gaillard, 1968). Syntypes in GB.

Distribution: coast of Australia from Warrnambool, Victoria, around south-eastern Australia to the mid north coast of New South Wales, and the north coast of Tasmania; Norfolk Island; San Ambrosio and San Felix, Juan Fernandez Islands, Chile.

According to Womersley (1987) the record by Lindauer *et al.* (1961) of *Padina fraseri* from the Kermadec Islands of New Zealand is more likely *P. gymnospora*, and the report by Taylor (1966) from the Philippines is likely to be of a different species. Adams (1994) also reported *P. fraseri* from the Kermadecs, but the plants were small and sterile.

References: Womersley, 1967, p. 222; Gaillard, 1968; Allender & Kraft, 1983, p. 88, fig. 6 D; Womersley, 1987, p. 217, pl. 2, fig. 1; figs 73D, 75D-G; Farrant & King, 1989, p. 390, fig. 14; Adams, 1994, p. 89, pl. 25.

**16) *P. glabra* Gaillard, 1966, p. 222, figs 1-3.**

Type locality: Dakar, Senegal, West Africa. Holotype presumably in PC but not located.

Distribution: West Africa and India.

References: Silva *et al.* (1996).

**17) *P. gymnospora* (Kütz.) Sonder, 1871, p. 47.**

Basionym: *Zonaria gymnospora* Kütz., 1859, p. 29, pl. 71, fig. II.

Type locality: St. Thomas, U.S. Virgin Islands, Caribbean. Holotype in MEL (Allender & Kraft, 1983).

Distribution: Caribbean, Caribbean Mexico, West Africa, Hawaii, western Australia.

Tax. syn.: *P. variegata sensu* Børgesen, 1914, p. 205; *P. vickersiae* Hoyt in Howe, 1920, p. 595 (type locality: Fort Macon jetty, Beaufort, North Carolina, USA) (*fide* Allender & Kraft, 1983); *P. howeana* Børgesen, 1920, p. 442.

References: Vickers, 1908, p. 37, pl. VIII (as *P. variegata*); Borgesen, 1914, p. 205, figs 157-161 (as *P. variegata*); Hoyt, 1920, p. 456, fig. 22; pl. XCII, figs 1 & 2; pl. CXIV, figs 1-3; Taylor, 1960, p. 236, pl. 34, fig. 1 (as *P. vickersiae*); Earle, 1969, p. 172, fig. 61 (as *P. vickersiae*); Schnetter, 1976, p. 77, pl. IX, fig. G; pl. XII, fig. A (as *P. vickersiae*); Allender & Kraft, 1983, p. 87, fig. 7E; Lawson & John, 1987, p. 131, pl. 16, fig. 5 (as *P. vickersiae*); Womersley, 1987, p. 217, figs 73C, 75A-C; Littler *et al.*, 1989, p. 112; Mateo-Cid & Mendoza-González, 1991, p. 78; Littler & Littler, 1997, p. 79, fig. 100.

**18) *P. haitiensis* Thivy in Taylor, 1960, p. 632 (& 235), pl. 75, fig. 1.**

Type locality: Isla Tortuga, Hispaniola. Holotype in MICH.

Distribution: Hispaniola and Turks Islands, Caribbean, Caribbean coast of Mexico and Colombia.

References: Schnetter, 1976, p. 74, pl. X, fig. B; Mateo-Cid & Mendoza-González, 1991b, p. 78; González-González *et al.*, 1996, p. 94.

**19) *P. japonica* Yamada, 1931, p. 69, pl. XIX, fig. 2.**

Syntype localities: Mikawa Prov., Iyo Prov., Nagato Prov., Higo Prov., Satsuma Prov., Echigo Prov., Japan. Holotype presumably in SAP.

Distribution: Japan, Taiwan, Caroline Islands.

Gaillard (1975) offered evidence to treat this taxon as conspecific with *P. sanctae-crucis*, but subsequent workers have continued to recognize it.

References: Okamura, 1932, p. 87, pl. 294, figs 1-4; Tanaka & Nozawa, 1962, p. 182, fig. 3; Trono, 1969, p. 36; Magruder & Hunt, 1979, p. 49; Lewis & Norris, 1987, p. 12; Kajimura, 1994, p. 72.

**20) *P. jonesii* Tsuda, 1972, p. 98, pl. 5, fig. 3.**

Type locality: Dougle Reef, Guam. Holotype in US.

Distribution: Guam; Xisha Islands (Guangdong Province, China).

References: Tseng, 1983, p. 198, pl. 100, fig. 4.

**21) *P. melemele* Abbott, 1996, p. 143, figs 1-3.**

Type locality: Ilio Point, Moloka'i Island, Hawaii. Holotype in BISH.

Distribution: also known from O'ahu Island, Hawaii.

**22) *P. mexicana* Dawson, 1944, p. 231, pl. 52, fig. 2.**

Type locality: Turner's Island reef, off Tiburon Island, Gulf of California, Mexico. Holotype in LAM.

Distribution: Pacific Mexico, tropical West Africa.

Reference: Lawson & John, 1987, p. 131, pl. 15, figs 8a-c; Mateo-Cid & Mendoza-González, 1991a, p. 24; González-González *et al.*, 1996, p. 156.

**23) *P. minor* Yamada, 1925, p. 251, fig. V.**

Type locality: Garanbi, Taiwan. Holotype presumably in SAP.

Distribution: Taiwan, Guam, Fiji, Japan, China, the Philippines, Papua New Guinea, Arabian Gulf.

References: Okamura, 1932, p. 56, pl. 279, figs 6-9; Chihara, 1970, p. 24, pl. 12(3); Tsuda, 1972, p. 97, pl. 5, fig. 2; Kapraun & Bowden, 1978, p. 200; Trono & Ganzon-Fortes, 1980; Tseng, 1983, p. 200, pl. 101, fig. 1; Verheij & Prud'homme van Reine, 1993, p. 429, fig. 5d, pl. 10: 7; Coppejans *et al.*, 1995, p. 184; De Clerck & Coppejans, 1996, p. 230, figs 49, 52; Calumpong & Meñez, 1997, p. 126.

**24) *P. pavonica* (Linnaeus) Thivy in Taylor, 1960, p. 234.**

Basionym: *Fucus pavonicus* Linnaeus, 1753, p. 1162.

Type locality: "In Mari Europae australis". Holotype presumably in LINN.

Thivy's binomial replaced the incorrect name *P. pavonia* (Linnaeus) Lamouroux (1816), which was based on *Fucus pavonius* Linnaeus, 1759, p. 1345.

Distribution: England, Atlantic coast of France and Spain, Mediterranean, Azores, Canary Islands, Bermuda and Caribbean; widely distributed in Indian Ocean

Tax. syn.: *P. mediterranea* Bory de Saint-Vincent, 1827, p. 590; *P. oceanica* Bory de Saint-Vincent, 1827, p. 590 (*fide* J. Agardh, 1848).

References: Harvey, 1846-1851, pl. XCI; Reinke, 1878, p. 15, pl. 2, figs 18-22, pl. 3, pl. 4, figs 1-12; Hamel, 1939, p. 343, fig. 57, 1-III; Price *et al.*, 1979, p. 1-67; Schnetter & Bula Meyer, 1982, p. 66, pl. X, fig. K-M; Coppejans, 1983, pl. 55; Cabioch *et al.*, 1992, p. 165, fig. 178; Littler & Littler, 1997, p. 81, fig. 102; González-González *et al.*, 1996, p. 94.

**25) *P. perindusiata* Thivy in Taylor, 1960, p. 632 (& 235), pl. 75, fig. 2.**

Type locality: Southwest Channel, Dry Tortugas, Florida. Holotype in MICH.

Distribution: Dry Tortugas, Florida, Gulf of Mexico.

References: González-González *et al.*, 1996, p. 94.

**26) *P. plumbea* (Areschoug) Levring, 1940, p. 226-227, fig. 5.**

Basionym: *Zonaria plumbea* Areschoug, 1851, p. 25-26; *Chlanidophora plumbea* (Areschoug) Papenfuss, 1940, p. 204, fig. 5.

Type locality: Port Natal [= Durban], South Africa. Holotype presumably in S.

Distribution: South Africa.

**27) *P. profunda* Earle, 1969, p. 167, figs 62-68.**

Type locality: a site "in 60 m depth about 19 mi. offshore from Loggerhead Key, Dry Tortugas, Florida". Holotype in FH.

Distribution: Dry Tortugas, Florida, Gulf of Mexico, North Carolina.

References: Schneider & Searles, 1973, p. 202; 1991, p. 162, fig. 195.

**28) *P. ryukyuana* Y. P. Lee & Kamura, 1991, p. 92, figs 1-13.**

Type locality: Bisezaki, Okinawa, Ryukyus, Japan.

Distribution: Okinawa, Japan. Holotype in CNU.

**29) *P. sanctae-crucis* Børgesen, 1914, p. 201, figs 153 & 154.**

Type locality: St. Croix, U. S. Virgin Islands. Holotype in C.

Distribution: Caribbean, Gulf of Mexico, Pakistan, Bangladesh, Papua New Guinea, Australia.

References: Taylor, 1960, p. 237, pl. 34, fig. 2; Chapman, 1963, p. 23, fig. 18 (as *Dictyosphaera jamaicensis*); Earle, 1969, p. 171, fig. 59; Gaillard, 1975, p. 85, figs 1-4; Schnetter, 1976, p. 75, pl. X, fig. A; Womersley, 1987, p. 219, figs 74A, 75H-J; Littler *et al.*, 1989, p. 114; Coppejans *et al.*, 1995, p. 184, fig. 23; Littler & Littler, 1997, p. 81, figs 101 (as *P. jamaicensis*) and 103; González-González *et al.*, 1996, p. 94.

Tax. syn.: *P. jamaicensis* (Collins) Papenfuss, 1977, p. 272.

Basionym: *Dictyosphaera jamaicensis* Collins, 1901, p. 251.

Type locality: Manchioneal, Jamaica. Syntypes: Phycotheca-Boreali Americana 780; lectotype apparently not yet designated.

**30) *P. somalensis* Hauck, 1887, p. 45.**

Syntype localities: Scara and Lasgori, Somalia.

Distribution: Somalia. Holotype in L.

**31) *P. stipitata* T. Tanaka & Nozawa in Tanaka, 1960, p. 100, text-figs 8-10, pl. V, A, B.**

Syntype localities: Funauke, Iriomote Island, Ryukyu Islands; Koniya, Amami Islands, Japan. Syntype specimens presumably in KAG; lectotype not yet designated.



Distribution: Ryukyu Islands and Amami Islands, southern Japan.

References: Tsuda, 1991, p. 45.

32) *P. thivya* Doty & Newhouse, 1966, p. 139, as "*P. thivyi*" figs 1, 2 ■ & b.

Type locality: reef flat seaward of the Natatorium, Waikiki, Honolulu, Oahu, Hawaii.

Holotype in BISH.

Distribution: Hawaiian Islands.

References: Magruder & Hunt, 1979, p. 49.



## THE SOIL ALGA *JAAGIELLA ALPICOLA* VISCHER (CHLOROPHYTA), A NEW MEMBER FOR THE ORDER PLEURASTRALES

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**ABSTRACT** — *Jaagiella alpicola* Vischer is an edaphic alga that forms filaments and cell packets. The cells have a single nucleus and a plate-like parietal chloroplast without a pyrenoid. A metacentric mitotic spindle was observed during cell division; the parental cell wall contributes to the formation of the common transverse cell wall. Biflagellated zoospores were formed in groups of two or four within non-modified cells of the thallus; the parental cell wall does not contribute to the zoospore cell envelope. The zoospores were naked, almost flattened, globally asymmetric and contained a single chloroplast with an eyespot. The basal bodies showed counter-clockwise orientation and were connected by a striated proximal and distal connecting fibre; the flagellar roots consist of a cruciate  $(7(6+1)-2-7(6+1)-2)$  microtubular root system. The ultrastructural and reproductive features of *J. alpicola* suggest its inclusion in the order Pleurastrales.

**RÉSUMÉ** — *Jaagiella alpicola* Vischer est une algue édaphique qui forme des filaments et des paquets sarcinoïdes. Les cellules présentent un seul noyau et un chloroplaste pariétal en forme de plateau, sans pyrénoïde. Le fuseau mitotique est métacentrique; la paroi de la cellule mère contribue à la formation de la paroi transversale. Les sporocystes sont des cellules du thalle non modifiées, qui donnent deux ou quatre zoospores biflagellées; la paroi de la cellule mère ne contribue pas à la formation de la zoospore. Les zoospores sont nues, plus ou moins comprimées et asymétriques, et ont un seul chloroplaste qui présente un stigma. Les corpuscules basilaires présentent une disposition *counter-clockwise* (interprétée comme résultant d'une dérive dans le sens contraire des aiguilles d'une montre par rapport à une disposition ancestrale hypothétique). Ils sont connectés entre eux par deux fibres striées, l'une proximale et l'autre distale. Les racines flagellaires constituent un système microtubulaire cruciforme  $(7(6+1)-2-7(6+1)-2)$ . Les caractéristiques morphologiques et ultrastructurales de *J. alpicola* suggèrent l'inclusion de cette espèce dans l'ordre des Pleurastrales.

**KEY WORDS:** chloroplast division, green algae, *Jaagiella*, Microthamniales, Mitosis, Pleurastrales, ultrastructure, zoospore.

### INTRODUCTION

The genus *Jaagiella* (Chlorophyta) was established by Vischer (1960). Bourrelly (1972) placed the genus in the Chaetophoraceae.

The original strain was unfortunately lost (Gärtner, pers. comm.). However, *J. alpicola* Vischer was rediscovered (Vela & Hernández-Mariné, 1987). A morphological and ultrastructural study was done (Hernández-Mariné & Revilla-Estrach, 1989); the habitat, appearance, and the presence of naked and flattened zoospores suggested its classification in the Pleurastrrophyceae (Hernández-Mariné & Revilla-Estrach, 1989).

Based on differences in cell division and structures associated with flagellar apparatus, Melkonian & Berns (1983) and O'Kelly & Floyd (1984) suggested a group with distinctive features that was established as a class, the Pleurastrrophyceae (Mattox & Stewart 1984), including the orders Pleurastrales (with the genera *Pleurastrum*, *Microthamnion*, *Pseudotreboxia*, *Treboxia*, and *Friedmannia*) and Tetraselmidales (with *Tetraselmis* as the only genus). The Pleurastrrophyceae was in time expanded with the addition of other taxa, based on both non-molecular and molecular evidence (Deason & Floyd, 1987; Kantz *et al.*, 1990; Zechman *et al.*, 1990; Friedl & Zeltner, 1994; Steinköter *et al.*, 1994; Watanabe & Floyd, 1994; Friedl, 1995, 1996; and a revision by Bakker *et al.*, 1997) whereas the genus *Pleurastrum* was shown to be of multiple origins (polyphyletic) and, therefore, its species were accorded to different classes (Friedl, 1996). Moreover, the order Pleurastrales was proposed as order Microthamniales by Melkonian (1990) due to the relatively unspecialized zoospores in *Microthamnion*. Lacking the latin diagnosis, the Microthamniales has not been validly published, although it has been adopted for most of the specialists of this group (Friedl & Zeltner, 1994; Steinköter *et al.*, 1994; Bakker *et al.*, 1997). The order Pleurastrales was included in the class Ulvophyceae, because of the same basic flagellate-type apparatus (Sluiman, 1989). Combining molecular and non-molecular data, Kantz *et al.* (1990) found that the Pleurastrrophyceae have a closer relationship with the Chlorophyceae than with the Ulvophyceae, although both possess the counter-clockwise basal body orientation in flagellated cells. Ribosomal DNA sequence data suggest the monophyletic origin of the Pleurastrales (as Microthamniales), and as it forms an independent evolutionary lineage together with autosporic coccoid green algae, it was suggested to treat it as a separate class of green algae (Friedl, 1995). The name Treboxiophyceae was chosen for this class since the type species of the genus *Pleurastrum* was found as a member of the Chlorophyceae and because lichen algae, such as *Treboxia impressa* V. Ahmadjian may best represent this class (Friedl, 1995). The independence of the Treboxiophyceae seems clear and it is accepted that ultrastructural features of the zoospore and molecular data are both reliable and comparable for assessing phylogenetic relationships (Friedl & Zeltner, 1994; Lokhorst & Rongen, 1994; Friedl, 1995; Bakker *et al.*, 1997). The purpose of this investigation was to obtain more information on non-molecular features of the monospecific genus *Jaagiella* and contrast the structural and ultrastructural data with recent information on the Pleurastrales.

## MATERIAL AND METHODS

Isolates of *J. alpicola*, derived from enrichment culture from Cap Norfeu (Vela & Hernández-Mariné, 1987) were used. Cultures were grown either in liquid or on 2% agarized Bold Basal medium (BBM) (Nichols & Bold, 1965), at different pH values under a 12:12h light/dark regime, 15–27°C and a light intensity of 40–100  $\mu\text{E m}^{-2} \text{s}^{-1}$ . Zoospore production was attempted by doubling the concentration of phosphorous ( $\text{K}_2\text{HPO}_4$ ) in the BBM or by changing pH, temperature and light regimes. Material from young colonies

or groups with freshly liberated zoospores was collected depending on the type of cell to be studied, namely vegetative cells or zoospores.

A Nikon Optiphot light microscope and a NIKON FX-35DX photographic machine were used. For transmission electron microscopy two fixation methods were followed: (1) chemical fixation and (2) cryofixation followed by cryosubstitution. For chemical fixation, the samples were fixed with glutaraldehyde (2%) in cacodylate buffer for two hours at 4° C, washed in the same buffer, postfixed in 2% OsO<sub>4</sub> (in cacodylate buffer) for 90 min. at 4° C, and rinsed in the same buffer. Part of the chemically fixed material was critical point dried and observed under the scanning electron microscope (SEM). Cryofixation was performed by projection against a Cryoblock (Reichert-Jung) cooled with liquid nitrogen (-196° C) according to Escaig (1982). After cryofixation the samples were cryosubstituted at -90° C for three days in a homemade cryosystem (Quintana, 1994) using acetone with 1-3% osmium tetroxide and then processed as described by Porta & López-Iglesias (1998). For freeze-fracture, filaments were frozen in Freon. Freeze-fracturing was carried out in a Balzers 301 high vacuum freeze-etch unit (Balzers A6, Balzers, Lichtenstein) and the specimen was shadowed with platinum and coated with carbon. Three electron microscopes were used: 1) Philips EM 200, 2) HITACHI H800MT, and 3) HITACHI 600 scanning electron microscope.

## RESULTS

On solid BBM the heterotrichous thalli of *Jaagiella alpicola* developed into colonies consisting of richly branched filaments radiating out from irregularly sarcinoid packets (Fig. 1). The outer filaments were openly branched and consisted of cylindrical and sometimes slightly elongated cells, especially at apices. The sarcinoid packets were formed of subglobose cells. A ball-like mass of filaments was formed when cultured in liquid media.

The filaments and sarcinoid packets were covered by a thin gelatinous matrix. Each vegetative cell contained a single, medium size, plate-like parietal chloroplast lacking a pyrenoid. Scattered starch granules were present. Pyrenoglobuly were lacking. A large subspherical nucleus was usually located next to the chloroplast. Centrioles were never seen in the interphase of vegetative cells. Large vacuoles containing electron dense material and multivesicular bodies were sometimes visible. The cell wall had smooth contours in the young cells. During maturation some undulations appeared on the cell membrane, along the whole cell perimeter (Figs 2, 3). Sexual reproduction was not observed. Asexual reproduction occurred by fragmentation and by zoospores. The spherical innermost vegetative cells of the sarcinoid packets were the first to undergo zoosporogenesis, with no visible changes in size or shape (Fig. 4). Terminal cells sometimes remained in the vegetative condition despite the transformation of adjacent cells into a zoosporangium. The first sign of zoosporulation was cytoplasm separation from the cell wall, leaving an electron transparent fibrillar network. 2-4 zoospores were liberated by the rupture of the parent cell wall. No protocol was effective in inducing the production and release of the zoospores. Sometimes zoospores were produced after introducing an ageing culture into a fresh medium. Zoosporulation mainly occurred in spring, although having been grown in controlled culture conditions for fourteen years.

Upon cessation of motility the zoospores became spherical. Germination of

zoospores was unipolar, although they developed bipolarly soon afterwards. Two weeks after zoospore settlement the filaments were composed of 8-28 cells and sarcinoid packets were developed; intercalary cells were almost 9  $\mu\text{m}$  long and 7  $\mu\text{m}$  wide, shorter than the apical cells, which were about 14  $\mu\text{m}$  in length and 7  $\mu\text{m}$  in width. In ageing cultures, intercalary cells were globular and around 8  $\mu\text{m}$  in diameter.

Zoospores were about 4-6  $\mu\text{m}$  in length, naked, almost pyriform and bore two equal flagella. They were asymmetric; the surface of the ventral face was slightly concave, whereas the dorsal face was convex (Fig. 5). The zoospores were uninucleate. The nucleus was located in the anterior portion of the zoospore, close to the basal bodies. A simple, large, parietal chloroplast filled the posterior area. An extended A-type eyespot (Dodge, 1973) on the dorsal posterior side of the cell; it contained nearly 50 regular electron-dense hexagonal lipid globules, situated in the same plane. It lay beneath the chloroplast envelope membranes, causing a slight bulge at the edge of the chloroplast (Fig. 6). A pyrenoid was lacking. A single mitochondrion with lobes parallel to the ventral side of the cell extended from basal bodies to the surrounding chloroplast. Several membrane-containing vesicles were situated near the basal body. The equal flagella were naked. The axoneme ended in a hair-point (Fig. 5). The transition region (tr) was separated in two unequal parts by the transverse septum or diaphragm (D) (Figs 7, 8): a proximal (ptr) and a distal (dtr) region; the dtr region being twice bigger than the ptr one. The D was associated with both the dtr and ptr (Figs 7, 8). The basal body (bb) (calculated according to Melkonian, 1984), was around 300-500 nm long (Fig. 8). At the end of the cartwheel structure, the bb had a thickened electron-dense plate perpendicular to the axis of the flagella (Fig. 8) that closed the proximal end of each basal body. Two striated connecting fibres were present between the two bb (Fig. 9); the distal striated connecting fibre (dcf) wrapped them externally; the proximal striated connecting fibre (pcf) tied both bb next to their inner angle and was in contact with three triplets of each bb (Fig. 9). According to the basal body absolute orientation system (O'Kelly & Floyd, 1983), the bb of the two flagella showed counter-clockwise orientation (Fig. 10) or an 11-5 o'clock configuration (Fig. 11) (Melkonian & Berns, 1983; Mattox & Stewart, 1984). An X-2-X-2 cruciate microtubular flagellar root system (Moestrup, 1978) was present. The X roots, located on the inner side of the overlapping basal bodies, were constituted by 6+1 microtubules, the single one being in the innermost position. The R-2 roots are located on the outer side of each basal body (Figs 11, 12).

In vegetative cells, the chloroplast divided by an asymmetric constriction. Mitochondria, vacuoles containing medium electron-dense material and some microtubules (arranged parallel to the division plane) were in the area of constriction (Figs 13, 14). Ingrowth of the cell wall and nuclear division apparently took place immediately after chloroplast division. The plane of cell division was parallel to the chloroplast division plane. The centrioles were visible from preprophase. During prophase, centrioles apparently duplicated, the nuclear envelope disappeared, and an asymmetric cell membrane invagination was apparent at opposite poles of the cell; this invagination was larger at the pole containing the centrioles. During metaphase (Fig. 15), the duplicated centrioles were located in pairs on both sides of the cleavage furrow, which was marked by the development of the cell membrane invaginations, in the same plane as the chromosomes (a metacentric spindle). A network of microtubules was formed radiating out of the chromosomes in a plane perpendicular to the centrioles (Fig. 15). At the end of the division process the nuclear envelope was reconstituted around the two daughter nuclei, each located beside the cleavage furrow (Fig. 16).

## DISCUSSION

The morphological features of *Jaagiella alpicola* observed in the present study were consistent with Vischer's (1960) original description. Zoosporulation, not described by Vischer, was present. We did not observe akinetes, although according to Vischer's drawings, they could be mistaken for settled zoospores.

Zoospore ultrastructure is consistent with that in other genera of the Pleurastrales. The shape of *J. alpicola* zoospores was asymmetric as in *Pseudotreboxia* (Melkonian & Peveling, 1988). Like *Leptosira terrestris* Printz (as *Pleurastrum terrestre* Fritsch *et* John in Melkonian, 1981), it had a type A eyespot (Dodge, 1973). It also showed a little bulge at the eyespot belt as is described for *Microthamnion* (Watson & Arnott, 1973; Watson, 1975). The electron dense structure at the basal body basement was similar to a structure described elsewhere; it has been called either a "flagellar platform" in *Microthamnion* (Watson, 1975) or a "terminal cap" in *Myrmecia israeliensis* (Chantan. *et* H.C. Bold) Friedl (as *Friedmannia israelensis* Chantan. *et* H.C. Bold in Melkonian & Berns, 1983). The TR in *J. alpicola* is similar to that in *Pleurastroriscina* (Deason & Floyd, 1987) since the transverse septum was associated with both the dTR and pTR, and also to that of *Myrmecia israeliensis* (as *Friedmannia israelensis*, Melkonian & Berns, 1983) and *Microthamnion* (Watson, 1975), although in these two latter cases the D was attached to only one part of the TR. A system II fibre (Melkonian, 1980) forming a rhizoplast in *Microthamnion* (Watson, 1975) and in *Pleurastroriscina* (Deason & Floyd, 1987) were not observed in *Jaagiella alpicola*. However, as was previously reported for other Pleurastralean genera (Deason & Floyd, 1987; Melkonian & Peveling, 1988), small deviations in zoospore ultrastructure are not considered taxonomically important.

The division process in vegetative cells of *J. alpicola* was consistent with that of some species of the Microthamniales. *J. alpicola* had a typical filament-type chloroplast division apparatus (Tewinkel & Volkmann, 1987), similar to that described for *Treboxia* (Chida & Ueda, 1991). However, this process was not synchronous with nuclear division but was followed by it.

The vegetative interphase cells that developed during thallus growth, did not have centrioles appearing at the onset of cell division in the same position as in *Leptosira erumpens* (Deason *et* H.C. Bold) Lukešová (Lokhorst & Rongen, 1994). In addition, the parental cell wall contributed to the formation of the common transverse cell wall. This division pattern is considered to be vegetative cell division (Ettl, 1988; Stuijman *et al.*, 1989) rather than "Sporulation" (*loc. cit.*). Moreover, the beginning of the cell division was different depending on the end product. In the process that ended in zoospore formation the parental cell wall did not contribute to the zoospore cell envelope. Thus both cytokinetic events were present in *J. alpicola*, as they are in *L. erumpens* and *Microthamnion* (Lokhorst & Rongen, 1994; Bakker *et al.*, 1997).

Because the present specimen of *J. alpicola* possesses a metacentric spindle and two flagella arranged in a counter-clockwise orientation, with two alternating types of microtubular roots in a cruciate pattern, it was included in the ultrastructurally coherent order Pleurastrales. Since we do not have molecular data, we have been unable to include it into the class Treboxiophyceae *sensu* Friedl (1995).

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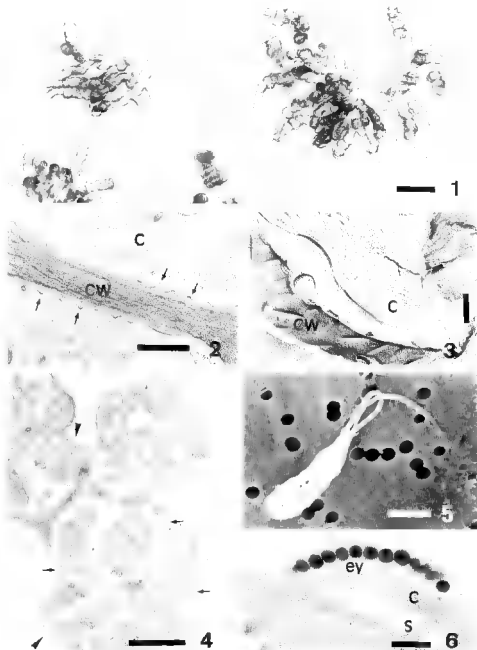
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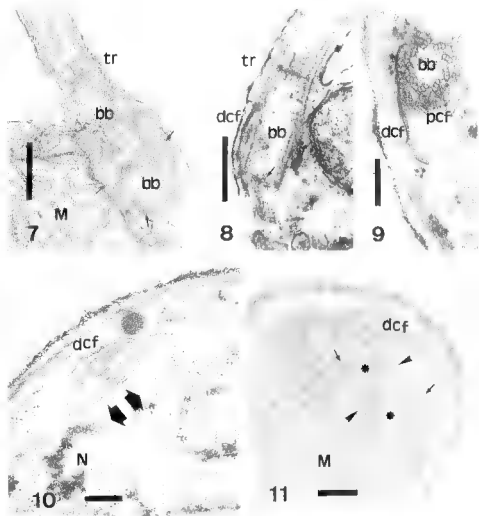
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Figs 1-6. Fig. 1. Light microscopy. Thallus formed by filaments and dense sarcinoid packets. Scale bar = 10  $\mu$ m. Fig. 2. Cell wall of a vegetative cell. Undulations on the cell membrane (arrows). Scale bar = 0.5  $\mu$ m. Fig. 3. Freeze-fracture of a vegetative cell showing the tracks on the cell wall. Scale bar = 0.3  $\mu$ m. Fig. 4. View of the thallus with cells developed into zoosporangia, revealing four zoospores inside each. Cells are dividing without ingrowth of the cell wall (arrowheads). Note the gelatinous matrix covering the thallus (arrows). Scale bar 5  $\mu$ m. Fig. 5. SEM view of a free zoospore. The flagella ended in a hair-point (arrowhead). Scale bar 2  $\mu$ m. Fig. 6. Transverse section in the eyespot region. Notice slight bulge in the plasma membrane. Scale bar = 0.25  $\mu$ m. C, chloroplast; CW, cell wall; ey, eyespot; S, starch granules.



Figs 7-11. Zoospore flagellar apparatus. Fig. 7. Anterior end of a zoospore section. Notice the flagellar root microtubules (arrows). Scale bar = 0.25  $\mu$ m. Fig. 8. Longitudinal section of the proximal region. The transition region, the basal body, the distal connecting fibre and the flagellar platform (arrow) are visible. Scale bar = 0.25  $\mu$ m. Fig. 9. Transverse section through a basal body showing the proximal and distal striated connecting fibres. Scale bar = 0.25  $\mu$ m. Fig. 10. Transverse section through the basal bodies showing its counter-clockwise orientation (arrows indicated the arrangement of the triplets of each basal body). Note also the distal striated connecting fibre. Scale bar = 0.25  $\mu$ m. Fig. 11. Nearly longitudinal section through the basal bodies (asterisks). The flagellar roots are shown: R-X roots (arrowheads), R-2 roots (arrows). Scale bar = 0.25  $\mu$ m. bb, basal body; dcf, distal connecting fibre; M, mitochondria; N, nucleus; pcf, proximal connecting fibre; tr, flagellar transition region.

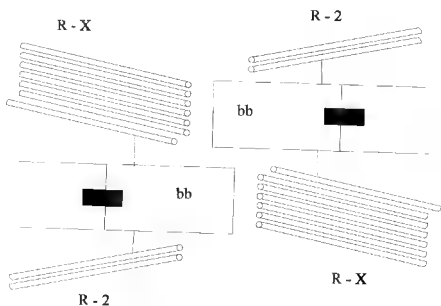
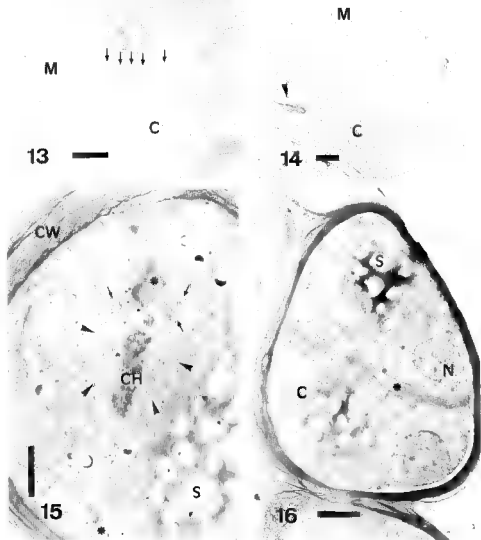
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Fig. 12. Schematic drawing of the overlapping basal bodies and the flagellar roots.  
bb, basal body.



Figs 13-16. Division processes of the vegetative cells. Fig. 13. Isthmus region in a dividing chloroplast. Arrows indicate the transverse microtubules. Scale bar = 0.5  $\mu$ m. Fig. 14. Vegetative cell at the end of a chloroplast division. Note the initial cell division process indicated by the presence of an infurrowing septum (arrowhead). Scale bar = 1  $\mu$ m. Fig. 15. Section of a dividing cell at metaphase to show the centrioles (small arrows), the chromosomes, the microtubules of the mitotic spindle (arrowheads) and the infurrowing septum (asterisks). Scale bar = 1  $\mu$ m. Fig. 16. Cytokinesis. The cleavage furrow (asterisk) was completed. Scale bar = 1  $\mu$ m.

C, chloroplast; CH, chromosomes; CW, cell wall; M, mitochondria; N, nucleus; S, starch granules.



## COVER ESTIMATES OF EPIPHYTIC CORALLINE ALGAE (CORALLINALES, RHODOPHYTA): BRAUN-BLANQUET VS COMPUTER IMAGE ANALYSIS

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**ABSTRACT** — Two techniques for examining percentage cover of epiphytic nongeniculate coralline algae were investigated. Percentage cover of *Pneophyllum fragile* Kützinger, *Melobesia membranacea* (Esper) Lamouroux and *Melobesia rosanoffii* (Foslie) Lemoine growing on *Laurencia elata* (C. Agardh) Hooker & Harvey (Rhodomelaceae, Ceramiales), was estimated using a modified Braun-Blanquet rating scheme and with computer image analysis (digitised cover method: DCM). Using an artificial paper pattern DCM was found to be accurate in assessing known "percentage cover". Cover of epiphytic nongeniculate corallines was more accurately determined by DCM than by the Braun-Blanquet rating system. The Braun-Blanquet method consistently yielded over-estimates when cover was high, and under-estimates when it was low. Using DCM rather than the Braun-Blanquet method, percentage cover is measured, not estimated. DCM may have broader applications for both non-algal epiphytes and non-epiphytic systems.

**RÉSUMÉ** — Deux techniques de détermination du pourcentage de couverture de corallines non articulées épiphytes ont été comparées. Les pourcentages de couverture de *Pneophyllum fragile* Kützinger, *Melobesia membranacea* (Esper) Lamouroux et *Melobesia rosanoffii* (Foslie) Lemoine épiphytes de *Laurencia elata* (C. Agardh) Hooker & Harvey (Rhodomelaceae, Ceramiales), ont été estimés, à l'aide d'un système de codage de Braun-Blanquet modifié, d'une part, et à l'aide d'un système d'analyse d'image par ordinateur (*Digitised Cover Method*: DCM), d'autre part. En utilisant une configuration artificielle en papier, le système DCM s'est révélé performant puisqu'il a déterminé les "pourcentages de couvertures" connus. La couverture des corallines non articulées épiphytes a été déterminée avec plus de précision par la technique du DCM qu'avec l'aide du système de Braun-Blanquet. La méthode de Braun-Blanquet a mené systématiquement à des surestimations lorsque le pourcentage de couverture était élevé et à des sous-estimations lorsque ce pourcentage était faible. Lorsque l'on utilise la méthode du DCM, le pourcentage de couverture est mesuré et non estimé. La méthode du DCM pourrait avoir des applications plus larges pour des épiphytes autres que des algues et pour des organismes non épiphytes.

**KEY-WORDS:** Braun-Blanquet, digitised cover method (DCM), epiphyte, image analysis, *Laurencia*, nongeniculate coralline algae, percentage cover, *Pneophyllum*, *Melobesia*.

## INTRODUCTION

Nongeniculate coralline algae (Corallinaceae, Rhodophyta) commonly occur as epiphytes on various marine plants worldwide (Steneck, 1986; Woelkerling, 1988; Kjøstørud, 1997). Studies of their ecology generally involve analysis of changes or patterns in their percentage cover of the substrate. So far, cover has been estimated using various modifications of the subjective rating system developed by Braun-Blanquet (1928) (e.g. Ballantine, 1979; Jacobs *et al.*, 1983; Heijs, 1985; Kendrick *et al.*, 1988; Otero-Schmitt & Pérez-Cirera, 1996). This method has advantages of speed (Kershaw, 1973; Kendrick *et al.*, 1988), but it is a scale which lacks measurement (Mueller-Dombois & Ellenberg, 1974). Furthermore, depending on the level of cover, inaccuracy and unconscious bias may limit its usefulness (Kershaw, 1973; Greig-Smith, 1983).

Recently Morcom *et al.* (1997) used image analysis to measure percentage cover of nongeniculate corallines on *Laurencia elata*. This, however, has raised unanswered questions such as: does image analysis have advantages and/or limitations when compared with Braun-Blanquet schemes, and is image analysis more accurate than Braun-Blanquet estimates?

In this study, Braun-Blanquet and computer image analysis (the digitised cover method: DCM) are assessed, first by using artificial paper patterns to determine the accuracy of the digitised cover method, and second, by comparing Braun-Blanquet and DCM estimates of cover in a natural system — nongeniculate corallines epiphytic on the red alga *Laurencia elata* (C. Agardh) J.D. Hooker & Harvey (Rhodomelaceae, Ceramiales).

## MATERIALS AND METHODS

To determine the accuracy of the digitised cover method (DCM) we compared digitised estimates of percentage cover with known percentage cover values (0.9%, 4.5%, 14%, 17%, 32% and 100%). Known percentage cover values were obtained using an artificial paper pattern. This was produced by cutting irregular shapes from paper, weighing them and calculating their percentage cover, then photocopying the pieces in a haphazard arrangement.

Two methods were used to assess the cover of epiphytic corallines. A modified Braun-Blanquet scheme was applied as follows. Percentage cover was estimated as: +, < 1%; 1 — ≥ 1% cover < 5%; 2 — ≥ 5% cover < 10%; 3 — ≥ 10% cover < 20%; 4 — ≥ 20% cover < 40%; 5 — ≥ 40% cover < 60%; 6 — ≥ 60% cover < 80%; 7 — ≥ 80% cover = 100%. Three experienced experimental volunteers and the first author performed the Braun-Blanquet assessments. DCM measurements were taken by the first author.

Percentage cover was digitised using the computer image analysis package Trace (Leading Edge Pty Ltd ©). DCM involves microscopic examination with computer image analysis. When substratum (or artificial paper pattern) is viewed through a binocular dissecting microscope (16x mag.) a red dot is visible through one eyepiece. This dot is the "marker point" of a mouse attached to a digitising tablet (Summa Sketch II ®, Summa-



graphics<sup>®</sup>). The red dot is used to trace the edges of plaques of corallines (or simulated epiphytic cover), and the results are displayed on an adjacent monitor. Percentage cover was measured by tracing the whole area being examined (i.e. paper area or 1 cm length of *L. elata*) and recording its value, then tracing the areas covered by each coralline species (or simulated cover) within this 1 cm length or paper area and dividing by the total area.

*Laurencia elata* (C. Agardh) J.D. Hooker & Harvey (Rhodomelaceae, Ceramiales) is a red alga found along southern Australian rough-water coasts (for further details see Morcom *et al.*, 1997). Axes of *Laurencia elata* were randomly selected for analysis from material from one locality during February 1994: Number 16 Reef (38°25'12"S, 144°49'00"E) Rye, Victoria. This data set is independent of that analysed in Morcom *et al.* (1997).

A total of eighty 1 cm lengths of *L. elata* were examined by three experimental volunteers plus the first author. Both sides of two fronds were examined (two replicates). Each person examined twenty 1 cm lengths, and each 1 cm length was examined by two people.

Three species of nongeniculate coralline algae were found on *Laurencia elata* fronds: *Pneophyllum fragile* Kützinger, *Melobesia membranacea* (Esper) Lamouroux and *M. rosanoffii* (Foslie) Lemoine. Within the genus *Melobesia*, most plants were *M. membranacea*, but some were *M. rosanoffii*. Microscopic sectioning is required to distinguish between the two species, so results from the two were pooled; in the remainder of this paper they are referred to as *Melobesia*.

Species identification follows Saito & Womersley (1974) for *Laurencia elata* and Penrose (1996) and Woelkerling (1996) for the nongeniculate corallines. Permanent slide collections and voucher specimens (LTB 17293) are housed at LTB (Department of Botany, La Trobe University, Bundoora, Victoria, Australia), and will eventually be transferred to MEL (National Herbarium of Victoria, South Yarra, Victoria, Australia).

## RESULTS AND DISCUSSION

The digitised cover method (DCM) accurately assessed known percentage cover ( $r^2 = 0.997$ , Fig. 1). Percentage cover estimates using DCM show a very close fit to the known cover values.

DCM showed that nongeniculate coralline algae covered 61% (*Pneophyllum* 49% and *Melobesia* 12%) of the total surface of *Laurencia elata*, non-coralline epiphytes covered 3% and the remaining 36% was unoccupied. *Pneophyllum* and *Melobesia* constitute the total coralline cover of *Laurencia elata*, where *Pneophyllum* dominates and *Melobesia* occurs sporadically. Cover within sampling units varied from absent to highly abundant, in both *Pneophyllum* (0–94%) and *Melobesia* (0–50%).

Percentage cover estimates of *Pneophyllum* and *Melobesia* (Figs 2, 3) varied between the two techniques. Comparative percentage cover estimates of *Pneophyllum* (Fig. 2) showed that below 60% cover, 95% of Braun-Blanquet estimates were lower than the DCM estimates for the same section of substrate; for estimates above 60% cover, all Braun-Blanquet estimates exceeded digitised cover estimates. Despite its infrequent occurrence, the percentage cover estimates of *Melobesia* (Fig. 3) were similar to those of *Pneophyllum*: most Braun-Blanquet estimates of percentage cover below 60% were lower than digitised cover estimates. These results highlight one of the failings of subjective rating systems, that of over- and under-estimating percentage cover (Greig-Smith,

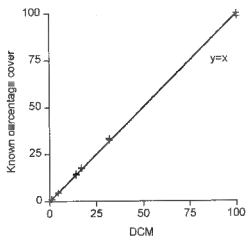


Fig. 1. Scatter plot of known percentage cover and DCM measured percentage cover. (DCM = the digitised cover method,  $n = 48$ ; weighed paper).

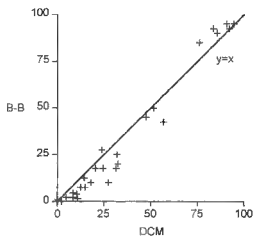


Fig. 2. Scatter plot of mean percentage cover of *Pnecophyllum* (DCM = digitised cover method, B-B = Braun-Blanquet method;  $n = 40$ ).

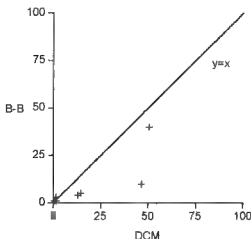


Fig. 3. Scatter plot of mean percentage cover of *Melobesia* spp. (DCM = digitised cover method, B-B = Braun-Blanquet method;  $n = 40$ ).

Table 1. The means and variances of the proportions cover of *Pneophyllum* and *Melobesia* estimated using DCM and B-B, and their ratios (DCM = digitised cover method, B-B = Braun-Blanquet,  $n = 40$ ).

Species	Mean			Variance		
	DCM	B-B	B-B/DCM	DCM	B-B	B-B/DCM
<i>Pneophyllum</i>	0.348	0.314	0.902	0.098	0.120	1.224
<i>Melobesia</i>	0.143	0.093	0.650	0.040	0.020	0.500

1983). Moreover when "species vary in conspicuousness, it is difficult to avoid overrating conspicuous species and underrating inconspicuous ones" (Greig-Smith 1983, p. 3).

Table 1 shows that overall the Braun-Blanquet method underestimated cover by 9.8% for *Pneophyllum* and 35% for *Melobesia*. It also underestimated the variance by 50%. This result (cover), particularly for *Melobesia*, may reflect small plaque size coupled with a sporadic distribution pattern and the associated difficulty of conspicuous species and inconspicuous ones (see Fig. 3).

When measuring cover of nongeniculate corallines, the digitised cover method was more accurate than the Braun-Blanquet method and thus eliminated much of the associated subjective error. However, there are limitations associated with DCM, including the time taken, and the "suitability" of the epiphyte and substratum. Using DCM was time-consuming (1 cm/5 mins), but improved accuracy and the ability to measure rather than to estimate percentage cover may remove "time" as a limitation. When using

DCM, some variability was observed when measuring cover repeatedly on lengths. Potential difficulties associated with cylindrical substrates have yet to be assessed.

By using DCM rather than the Braun-Blanquet approach, percentage cover is measured and not estimated. Conclusions drawn from the Braun-Blanquet data should be considered with caution (see Table 1, means & variances). Analysis using DCM appears useful for encrusting epiphytes on seagrasses or on algae with a more or less flat surface.

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## CHEMICAL COMPOSITION OF THE GREEN ALGA *BOTRYOCOCCUS BRAUNII*

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**ABSTRACT** — The growth and chemical composition of the green alga *Botryococcus braunii* grown in a modified and improved basal medium under batch conditions have been examined and compared with composition of the protein-rich filamentous cyanobacterium *Spirulina platensis*. The growth rate and biomass yield of the alga was enhanced to two fold in the modified medium while the profiles of amino acids and lipids (saturated and unsaturated fatty acids) remained almost unaffected. The results are discussed with respect to carbohydrate, protein, amino acids, lipid and fatty acid composition of *Botryococcus braunii* in improved medium in relation to its economic importance.

**RÉSUMÉ** — La croissance et la composition chimique de l'algue verte *Botryococcus braunii*, cultivée en batch dans un milieu de base modifié et amélioré, ont été examinées. Sa composition chimique a été comparée à celle de la cyanobactérie filamenteuse riche en protéines *Spirulina platensis*. Le taux de croissance et le rendement en biomasse de *B. braunii* est deux fois meilleur dans le milieu modifié, tandis que les profils en acides aminés et en lipides (acides gras saturés et insaturés) restent presque inchangés. Les résultats concernant la composition en hydrates de carbone, protéines, acides aminés, lipides et acides gras de *B. braunii* dans le milieu amélioré sont discutés au regard de l'importance économique de cette algue. (Traduit par la Rédaction)

**KEY WORDS:** *Botryococcus braunii*, Chlorophyta, culture medium, chemical composition, Cyanophyta, cyanobacterium, microalga, *Spirulina platensis*.

## INTRODUCTION

The production of photosynthetic biomass via reduction of atmospheric CO<sub>2</sub>, is a promising source of food and energy, since it is renewable. In aquatic ecosystems, algae are the major biomass producers, and for these reasons, considerable attention has been paid to the exploitation of the potentials of micro algae as food, feed and fuel (Shelef & Soeder, 1980; Becker, 1993). Amongst the various species, the filamentous cyanobacterium *Spirulina platensis* (Nordstedt) Geitler is an attractive source of single cell protein of a high nutritive value (Ciferri, 1983). However, the major disadvantage is the requirement of high nitrate nitrogen and alkaline medium for growth (Zarrouk, 1966). The green

colonial alga *Botryococcus braunii* Kützinger occupies a unique position in being a rich source for production of hydrocarbons, and total lipids. It also is supposed to be ancestral for the origin of Boghead coals and natural rubbery deposit, the coorongite (Largeau *et al.*, 1980; Chirac *et al.*, 1985). Vegetative cells of *B. braunii* may accumulate unusually high levels of lipids and hydrocarbon rich lipids, amounting to 30-70% of its dry weight under different conditions of growth (Wolf, 1983; Yamaguchi *et al.*, 1987). This high production of hydrocarbons seems to influence the growth and total biomass productivity adversely (Belcher, 1968; Casadevall *et al.*, 1985). Therefore, numerous attempts have been made in the past to develop suitable media for growing *B. braunii* under alternative conditions for sustained production of algal biomass with higher quantities of hydrocarbons (Sawayama *et al.*, 1992, 1994). In this communication we report the growth of the alga in modified, improved, basal medium under batch conditions and compare the chemical composition, fatty and amino acid profiles of *Botryococcus braunii* with those of *Spirulina platensis*.

## MATERIALS AND METHODS

The colonial green alga *Botryococcus braunii* UTEX 572 and the filamentous blue green-alga (cyanobacterium) *Spirulina platensis* were obtained from Austin culture collection, USA and from Prof. Ripley D. Fox, France, respectively. *S. platensis* was grown in a modified Zarrouk's medium (1966) while *Botryococcus braunii* was grown in a modified, improved, medium that of Chu No. 10 (Safferman & Morris, 1964) having the following composition of macro elements  $\text{KNO}_3$  (0.4 g l<sup>-1</sup>),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.05 g l<sup>-1</sup>),  $\text{K}_2\text{HPO}_4$  (0.05 g l<sup>-1</sup>),  $\text{Na}_2\text{CO}_3$  (0.04 g l<sup>-1</sup>),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.075 g l<sup>-1</sup>),  $\text{C}_6\text{H}_5\text{O}_7 \cdot \text{Fe} \cdot 5\text{H}_2\text{O}$  (0.0035 g l<sup>-1</sup>),  $\text{Na}_2\text{EDTA}$  (0.0035 g l<sup>-1</sup>) and 1 ml of micro element solution per litre was added containing  $\text{H}_3\text{BO}_3$  (2.86 g l<sup>-1</sup>),  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  (1.8 g l<sup>-1</sup>),  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (0.22 g l<sup>-1</sup>),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.079 g l<sup>-1</sup>),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (0.04 g l<sup>-1</sup>),  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (0.025 g l<sup>-1</sup>) and medium was also supplemented with 5% (v/v) soil extract (Rai *et al.*, 1987). Algal cultures were grown in a culture room maintained at  $24 \pm 1^\circ\text{C}$  and illuminated at an intensity of  $40 \mu\text{mol m}^{-2} \text{s}^{-1}$  (16 h/8 h light/dark cycle).

The growth of the algae was determined via dry weight measurements and total chlorophyll content extracted in methanol according to MacKinney (1941). For analysis of chemical constituents, the algal samples were harvested, repeatedly washed to remove salts and dried in oven at  $60^\circ\text{C}$  for 6-8 h before further use. The total protein content of the algae was determined by folin-phenol reagent (Lowry *et al.*, 1951), carbohydrate content by phenol-sulphuric acid reaction (Dubois *et al.*, 1956) and lipids by acid dichromate method (Amenta, 1964). The hydrocarbons in the algal cells of stationary phase were extracted from the freeze-dried cells by sonication (MSE Soniprep 150) with hexane and estimated according to Maxwell *et al.* (1968). To analyse amino acid composition, aliquots of 25 mg of dried, defatted and powdered samples were hydrolysed with 6 N HCl, flushed with nitrogen, for 24 h at  $104-110^\circ\text{C}$ . The hydrolysate was concentrated to dryness under vacuum and the residue was dissolved in 66 mM sodium citrate buffer (pH 2.2) and analysed on a LKB 4101 Amino acid analyser for amino acid profile. For fatty acid analysis, aliquots of the algal samples were saponified, methylated according to Cocks & Rede (1966) and analysed by gas chromatography as described earlier (Prakash & Pal, 1992). The data presented are average of three replicates of three independent experiments conducted under identical conditions.



## RESULTS AND DISCUSSION

The growth and chemical composition of *Botryococcus braunii* obtained in the basal and improved media in batch cultures are compared in Table 1. In the improved medium, calcium nitrate, sodium silicate and citric acid of the basal medium (Chu No. 10) were replaced with potassium nitrate, calcium chloride, sodium EDTA and soil extract. The final yield of total biomass obtained in the modified, improved, medium was 46% higher when compared to the basal medium. In the improved, modified, medium the generation time of the alga was reduced to half, and specific growth rate was increased over the values obtained in the basal medium. Chemical analysis showed relatively higher amounts of total protein (22-24%), lipids (44-46%) and chlorophyll (13.98 mg l<sup>-1</sup>) in the modified medium while carbohydrate level did not change significantly. The hexane fractions of the algal samples grown in basal and improved medium showed that hydrocarbons account for about 12 ± 1% and 17 ± 1% of total lipids respectively. Our data have confirmed previous results that in the improved medium, *Botryococcus braunii* is able to produce and accumulate large amount of lipids in the stationary phase of batch cultures (Ben-Amotz *et al.*, 1985; Fogg, 1988).

Table 1. Growth and chemical composition (dry wt %) of *Botryococcus braunii* grown in different media after 20 days of incubation.

	Basal medium	Improved medium + soil extract (5% v/v)	% increase in modified medium
Dry weight (g l <sup>-1</sup> )	0.78 - 0.82	1.14 - 1.18	46.0
Total chlorophyll (mg l <sup>-1</sup> )	10.90 - 11.00	13.98 - 14.25	28.0
Specific growth rate (μm b <sup>-1</sup> )	5.3 10 <sup>-2</sup>	10.2 10 <sup>-2</sup>	117.0
Doubling time (h)	130-132	68 - 70	91.0
Protein (%)	18 - 20	22 - 24	21.0
Carbohydrates (%)	10 - 13	12 - 14	12.5
Lipids (%)	40 - 42	44 - 46	10.5
Hydrocarbon* (% of total lipids)	11 - 13	16 - 18	42.0

\* Hydrocarbons were extracted from 30 days old culture. All the values are mean of three replicates of three independent experiments

The amino acid composition of *Botryococcus braunii* grown in the basal and the improved medium, along with *Spirulina platensis* and FAO standard (1973), are compared in Table 2. The levels of essential amino acids like threonine, valine methionine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine were relatively higher for *Botryococcus braunii* grown in the modified and improved medium compared to the basal medium. Despite the increase in protein content of the alga grown in the modified, improved, medium, the amino acid profile of the alga did not show large changes over the control except for arginine which was higher in the algal samples grown on the improved medium.

Table 2. Amino acid composition (g/16 g N) of *Botryococcus braunii* grown in different media and of *Spirulina platensis*.

Amino acids	<i>B. braunii</i>		<i>S. platensis</i> (Zarrouk's medium)	FAO Standard (1973)
	BM	IM		
<b>Essential</b>				
Thr	5.6	5.9	5.8	4.0
Val	6.2	6.5	8.8	5.0
Met	0.5	0.7	2.1	3.5
Ile	4.5	4.8	7.6	4.0
Leu	7.4	7.9	7.2	7.0
Phe	5.7	5.9	4.4	
Lys	3.7	3.9	5.0	5.5
His	1.2	1.4	1.9	
Arg	4.1	6.0	5.8	
<b>Non essential</b>				
Asp	10.1	9.9	8.4	
Ala	8.2	7.6	7.5	
Ser	4.5	4.4	5.6	
Glu	12.5	12.9	8.2	
Pro	5.1	5.1	5.4	
Gly	8.7	8.2	8.1	
Cys	1.0	0.8	0.4	3.5
Tyr	2.6	2.6	2.6	6.1

BM = Basal medium; IM = Improved medium + soil extract (5 % v/v).

Composition of some of the essential amino acids in *Botryococcus braunii* grown on the improved medium were similar to those of *Spirulina platensis* and the FAO standard for essential amino acids in plant proteins. The levels of most of the essential amino acids were quite balanced except for methionine, histidine and lysine.

Table 3 presents the comparison of fatty acid composition of *Botryococcus braunii* and *Spirulina platensis*. Amongst the saturated fatty acids, lauric (12:0), myristic (14:0) and stearic (18:0) acids were in low amounts while palmitic (16:0) was the major saturated fatty acid in both the algae although its percentage was about 2-fold higher in *S. platensis*. Oleic (18:1), linoleic (18:2) and linolenic (18:3) were the major unsaturated fatty acids in *Botryococcus braunii*. The level of oleic acid was 5.7-fold higher in *Botryococcus braunii* and the level of linoleic acid was 2.6 -fold higher in *Spirulina platensis*, while the level of linolenic acid was nearly the same in both the algae. The remaining fatty acids in *Botryococcus braunii* also showed marked differences with *Spirulina platensis*. The amount of total unsaturated fatty acids in *Botryococcus braunii* was about 2 — fold higher than in *Spirulina platensis*. The saturated to unsaturated fatty acid ratio and total level of unsaturated fatty acids showed that lipid quality in *Botryococcus braunii* was superior to that of *Spirulina platensis*, whereas no marked differences were observed in the fatty acid composition of *Botryococcus braunii* grown in the basal or improve media. These results on fatty acid profile in *Botryococcus braunii* are in close agreement with earlier reports by Douglas *et al.* (1969), Dubinsky *et al.* (1978) and Ben-Amotz *et al.* (1985).

Cyanobacteria and green plants (including eukaryotic green algae) differ in lipid composition and in the biosynthetic pathway of lipids. The former contains monogluco-

Table 3: Fatty acid composition of *Botryococcus braunii* and *Spirulina platensis*.

Fatty acid	% of total methylated fatty acid mixture		
	<i>B. braunii</i>		<i>S. platensis</i> (Zarrouk's medium)
	BM	IM	
Lauric acid (12:0)*	2.1	2.4	0.7
Myristic acid (14:0)	2.6	2.8	1.1
Tetradec-5-enoic acid (14:1)	0.4	0.3	0.6
Palmitic acid (16:0)	18.1	17.8	35.2
Hexadec-9-enoic acid (16:1)	1.3	1.2	8.5
Stearic acid (18:0)	1.6	1.7	0.6
Oleic acid (18:1)	38.3	38.6	6.8
Linoleic acid (18:2)	8.6	8.3	21.2
Linolenic acid (18:3)	20.8	20.6	19.8
Unidentified	6.2	6.3	5.5
Total unsaturated	69.4	69.0	37.6
Saturated : Unsaturated	0.35	0.35	0.66

\* Number of carbon: number of double bonds; BM = Basal medium; IM = Improved medium + soil extract (5 % v/v); unsat- unsaturated; sat- saturated.

syl, diacylglycerols which are synthesized by transfer of glucose unit from UDP-glucose to diacylglycerol (Sato & Murata, 1982). By contrast, green plants contain phosphatidylcholine directly synthesized by transfer of the galactose unit from UDP-galactose to diacylglycerol (Roughan & Slack, 1982). However, in *Botryococcus braunii* the interesting fact is the shift and diversion of photosynthetic driven reductant into the efficient synthesis and accumulation of saturated and unsaturated fatty acids and hydrocarbons. The slow growth rate of the alga has been attributed to changes in cellular structure, membrane fluidity and shift in physiological metabolism due to the accumulation of long-chain hydrocarbons botryococcenes (Wolf *et al.*, 1985; Fogg, 1988). By contrast, cyanobacteria have a very simple lipid composition, similar to the chloroplast of higher plants, which plays a central role in regulating membrane fluidity during thermoadaptation and growth (Golecki & Drews, 1982). This reflects a different composition and mechanism of lipid metabolism in *Botryococcus braunii* and *Spirulina platensis*. Therefore, in view of our studies, the colonial green alga *Botryococcus braunii* seems to offer an economically viable system for utilizing solar energy in the production of hydrocarbons due to its ability to accumulate a high lipid content with balanced amino acid profile and enhanced growth rate in the improved basal medium (Ben-Amotz *et al.*, 1985; Wolf *et al.*, 1985).

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## ANALYSE D'OUVRAGE

**Colin S. Reynolds, 1997 — Vegetation Processes in the Pelagic: A model for Ecosystem Theory.** Luhe, Germany: Ecology Institute, Oldendorf. Excellence in Ecology, vol. 9, XXVII + 371 p. DM 68 (environ 250 FF).

Colin S. Reynolds nous a habitué à de remarquables travaux en écologie du phytoplancton. Il est l'auteur du volume 9 de la prestigieuse série "Excellence in Ecology", fondée et éditée par O. Kinne, et ce livre ne déroge pas à cette règle de qualité. C.S. Reynolds a publié cet ouvrage en réponse au "Ecology Institute Prize" qui lui a été décerné en 1994; on attend en effet des candidats qu'ils synthétisent leurs résultats dans un ouvrage magistral. Dans cet excellent ouvrage, qui sera sans doute un outil pédagogique incontournable, l'auteur nous fait profiter de ses 25 ans d'expérience sur l'écologie du phytoplancton marin et d'eau douce. Le livre est présenté en neuf chapitres qui nous invitent, avec une grande clarté, à aborder les différents aspects de l'écologie et de la biodiversité du phytoplancton.

Après une introduction générale relative au phytoplancton marin et des eaux douces, l'auteur passe en revue, dans le premier chapitre, les différentes associations d'espèces phytoplanctoniques et leur signification écologique, en y introduisant sa propre conception, sans trop insister sur l'aspect systématique. Les propriétés physiques et chimiques du milieu pélagique ainsi que les interactions phytoplancton-environnement constituent le second chapitre. Les mécanismes d'adaptation des populations aux conditions de lumière et de disponibilité des nutriments, dont dépend la survie des espèces, sont très bien discutés dans le troisième chapitre. Nous y trouvons ainsi des rappels de biochimie moléculaire, des principes de base de la photosynthèse, jusqu'au rôle photoprotecteur des chloroplastes. Les aspects de la croissance des populations phytoplanctoniques et de production de biomasse, qui dépendent de la lumière et des ressources nutritionnelles du milieu, sont abordées dans le chapitre quatre. Dans le cinquième chapitre, C.S. Reynolds aborde avec beaucoup de dextérité toutes les stratégies adaptatives développées par les groupes fonctionnels écologiques en réponse à l'instabilité du milieu pélagique. Les capacités physiologiques des algues à s'adapter aux conditions extrêmes, leur ont permis et leur permettent encore de coloniser de nouveaux milieux. Taux de croissance, élaboration de biomasse, formes de résistance, motilité, taille et morphologie cellulaire sont considérés comme des éléments clé dans le succès des générations. Ces aspects interviennent dans la dynamique des populations phytoplanctoniques, la sélection des espèces ainsi que leur pérennité. Reynolds souligne par ailleurs les implications évolutives de ces adaptations. Le sixième chapitre est consacré à l'utilisation et aux pertes de la production primaire pélagique. Une partie de la biomasse produite est consommée instantanément par les herbivores et devient une source d'énergie pour la chaîne alimentaire et pour le maintien du système. Elle peut être également un support aux activités bactériennes et rejoint ainsi la "boucle microbienne". Des mécanismes physiques (sédimentation, turbulence...) sont aussi à l'origine d'importantes pertes de cette biomasse. L'écologie et l'organisation des communautés phytoplanctoniques dans les différents types de lacs sont abordées dans le chapitre sept qui constitue une synthèse sur les assemblages d'espèces algales, les successions écologiques et les variabilités inter-annuelles des communautés algales. L'auteur rappelle par ailleurs que la structure de ces communautés et la croissance des populations sont continuellement sujettes aux fluctuations de l'environnement pélagique. Le chapitre huit est une discussion du contrôle et de la régulation de ce système dont il montre la très grande complexité. C.S. Reynolds présente l'hypothèse des "Cascades trophiques" (Carpenter *et al.*, 1985), selon laquelle le contrôle se ferait uniquement à partir des niveaux supérieurs du réseau trophique. L'effet de la prédation se

répercute jusqu'au niveau inférieur de la chaîne trophique et aboutit au contrôle de la production primaire. Il discute l'hypothèse du contrôle "Bottom-up/top-down" des communautés pélagiques qui sont soumises à deux types de forces de régulation simultanées et non dissociables: descendante (*top-down*, par la prédation) et ascendantes (*bottom-up*, par les ressources). C.S. Reynolds expose également dans cette partie, les capacités d'auto organisation de l'écosystème pélagique et d'orientation des différents flux énergétiques.

Plutôt qu'une conclusion générale, l'auteur nous offre un dernier chapitre intitulé "Leçons et applications". Il nous invite à réfléchir sur les modalités de conservation des écosystèmes terrestres et aquatiques et sur la gestion de leurs ressources. Il souligne la fragilité de l'équilibre de l'écosystème, sa surexploitation (agriculture, pêche, déforestation) qui pourrait le faire basculer de manière irréversible vers un écosystème dont l'Homme serait exclu. L'exemple de l'eutrophisation des eaux est discuté. C.S. Reynolds expose sa vision négative de la technique de biomanipulation anthropique du réseau trophique utilisé pour le contrôle des biomasses algales. Les résultats controversés de cette technique le rendent sceptique quant à son efficacité comme outil d'aménagement et de restauration des plans d'eaux eutrophiés. Selon Reynolds, le maintien de la biodiversité et d'une richesse spécifique est la seule manière de stabiliser le fonctionnement de l'écosystème.

Cet ouvrage n'est donc pas seulement un exposé scientifique mais un essai philosophique sur l'écologie et ses nouveaux concepts. C'est une mine de connaissances et, de ce fait, une des meilleures références dans le domaine de la limnologie et, en particulier, sur l'écologie du phytoplancton.

Sahima Hamlaoui

Carpenter, S. R., Kitchell J.F. & J. R. Hodgson J.R., 1985 — Cascading trophic interactions and lakes productivity. *Bioscience* 35: 634-639.



## ANNONCE DE CONGRÈS

Colloque à la mémoire de  
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Professeur honoraire de l'Université Catholique de l'Ouest

jeudi 29 et vendredi 30 avril 1999  
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